$C_6H_5CONHCH_2CO_2H$ MW: 179.18 CAS: 495-69-2 RTECS: MR8150000
$CH_3C_6H_4CONHCH_2CO_2H$ 193.20 o-42013-20-7
m-27115-49-7
p-27115-50-0

METHOD: 8301, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 February 1984**
Issue 2: 15 August 1994

BIOLOGICAL INDICATOR OF: exposure to toluene and xylene.

SYNONYMS: hippuric acid: N-benzoylglycine.

SAMPLING		MEASUREMENT	
SPECIMEN:	urine, end of shift after 2 days exposure	**TECHNIQUE:**	HPLC-UV DETECTION
VOLUME:	complete spot voiding	**ANALYTE:**	(1) hippuric acid and (2) methyl hippuric acid
PRESERVATIVE:	a few crystals of thymol; keep @ 4 °C	**EXTRACTANT:**	ethyl acetate
SHIPMENT:	pack in styrofoam shipper with bagged refrigerant, ship by air express	**WAVELENGTH:**	254 nm
SAMPLE STABILITY:	stable 1 week @ 4 °C and 2 months @ -20 °C	**COLUMN:**	reverse phase (C_{18})
CONTROLS:	collect samples from unexposed, matched population as well as pre-shift urines from exposed workers	**MOBILE PHASE:**	90/10/0.02% (v/v/v) water/acetonitrile/glacial acetic acid; 3 mL/min
		CALIBRATION:	aqueous solutions of analyte
		QUALITY CONTROL:	frozen pooled urine; normalize to creatinine
		RANGE:	0.2 to 1.0 mg/mL
		ESTIMATED LOD:	(1) 0.015 µg/mL; (2) 0.030 µg/mL
		RECOVERY:	98%
		PRECISION ($\hat{S}_r$):	0.04

APPLICABILITY: Hippuric acid and m-methyl hippuric acid are the principal metabolites of toluene and xylene, respectively. An occupational exposure to either of these organic solvents may be monitored by following the pattern of excretion of thes e metabolites in urine.

INTERFERENCES: None known; however, para- and meta- isomers elute together in this system. There are other sources of hippuric acid such as food preservatives, ethylbenzene, and styrene.

OTHER METHODS: This is based on the method of Matsui, et al [1]. Method 8300 can be used for screening. Isotachophoresis has also been used [2]. Newer HPLC methodologies do a better job of resolving these compounds [3].

REAGENTS:

1. Thymol, USP.
2. Sodium chloride.
3. Hydrochloric acid, conc. (36% w/w).
4. Ethyl acetate, HPLC quality.
5. Hippuric acid stock solution, 1 mg/mL. Dissolve 100.0 mg hippuric acid in 75 mL distilled water. Dilute to 100 mL. Stable one month.
6. o-Methyl hippuric acid, 1 mg/mL. Prepare as described for hippuric acid.
7. m-Methyl hippuric acid, 1 mg/mL. Prepare as described for hippuric acid.
8. p-Methyl hippuric acid, 1 mg/mL. Prepare as described for hippuric acid.
9. Mobile phase. Add 900 mL distilled water to 100 mL acetonitrile (HPLC grade) and 200 µL glacial acetic acid. Mix and filter.
10. Prepurified nitrogen, 99.9%.

EQUIPMENT:

1. Bottles, polyethylene, 250-mL.
2. Refrigerant, bagged ("Blue Ice," or equivalent).
3. HPLC system consisting of sample injector, pump, column, ultraviolet detector at 254 nm, strip chart recorder, integrator, and column; plastic, 14 x 155-mm, packed with 10-µm irregularly-shaped reversed phase (C_{18}) packing.
4. Radial compression module capable of delivering constant, uniform pressure to the entire longitudinal surface of the column.
5. Concentrator-evaporator with heated waterbath.
6. Centrifuge.
7. Analytical balance.
8. Pipettes, serological, 10-mL, with pipet bulb.
9. Centrifuge tubes, glass-stoppered, graduated, 15-mL.
10. Culture tubes, 12-mm x 75 mm, disposable.
11. Micropipettes, 40-, 100- and 200-µL.
12. Microsyringe, 10-µL.

SPECIAL PRECAUTIONS: Samples of urine collected from humans pose a real health risk to laboratory workers who collect and handle these samples. These risks are primarily due to personal contact with infective biological samples and can have serious health consequences, such as infectious hepatitis, and other diseases. There is also some risk from the chemical content of these samples, but this is much less. Those who handle urine specimens should wear protective gloves, and avoid aerosolization of the samples. Mouth pipetting, of course, must be avoided.

SAMPLING:

1. Collect a spot sample of urine in a 250-mL polyethylene bottle containing a few crystals of thymol.
   NOTE: Take the sample at the end of the second day of suspected exposure to toluene or xylene. Also take pre-exposure samples and samples from non-exposed workers as controls.
2. Pack bottles in styrofoam shipper with bagged refrigerant and ship by air express.

SAMPLE PREPARATION:

3. Perform a creatinine determination on an aliquot of urine (e.g., [4]).
4. Pipette 1.0 mL of well-mixed urine into a 15-mL graduated centrifuge tube.
5. Add 40 µL conc. HCl, mix, and add 0.3 g sodium chloride (enough to saturate).
6. Add 4 mL ethyl acetate. Shake for 2 min.
7. Centrifuge at 100 x gravity for 5 min. Transfer 200 µL of the organic layer to a culture tube and evaporate to dryness using a heated waterbath and a gentle stream of nitrogen.
8. Redissolve the residue in 200 µL distilled water.

CALIBRATION AND QUALITY CONTROL:

9. Prepare working standards over the range 0.2 to 1 mg/mL by dilution of hippuric acid stock solution and methyl hippuric acid stock solutions with distilled water. Working standards are stable for 1 week at room temperature.
10. Prepare and analyze the working standards together with samples, blanks and controls (steps 4 through 8 and 14 and 15).
11. Determine the peak height to concentration ratio (H/C) using the mean peak height of three injections for each working standard. Calculate the response factor, F, for each compound by dividing the respective peak height to concentration ratio by the peak height to concentration ratio for hippuric acid.
12. Include a batch of replicate standards with each batch of samples.
13. Include aliquots of previously analyzed frozen urine pool.

MEASUREMENT:

14. Set up the HPLC according to manufacturer's recommendations and to conditions on page 8301-1.
15. Inject 5 µL of the test solution from step 8 into the HPLC. Determine peak heights.

CALCULATIONS:

16. Calculate the concentration of analyte in the urine sample, C_u (mg/mL), using the concentration of a standard, C_s (mg/mL), the peak height of the analyte, H, and the peak height of the respective standard, H_s:

$$C_u = \frac{C_s \cdot H}{F \cdot H_s}.$$

17. Calculate the concentration of analyte/g creatinine in the urine sample, C (g/g creatinine), using the concentration of creatinine in urine determined in step 3, C_r (g creatinine/L urine):

$$C = \frac{C_u}{C_r}.$$

GUIDES TO INTERPRETATION:

1. The upper limit of the range for hippuric acid in non-exposed NIOSH employees was 0.6 mg/mL (0.6 g/L). Meta-methyl hippuric acid is not found in non-exposed humans.
2. Lauwerys [5] reports an upper limit of normal for hippuric acid of 1.5 g/g creatinine using a specific gas chromatographic method. He reports a "tentative maximum permissible value" of 2.5 g/g creatinine.
3. Lauwerys [5] reports a "tentative maximum permissible value" of 1.5 g/g creatinine for methyl hippuric acid collected at the end of a work shift.

EVALUATION OF METHOD:

Within-run precision ($\bar{S}_r$) averaged 0.04 over the analytical range. No further evaluation of the method was performed except as reported by Matsui, et al. [1].

REFERENCES:

[1] Matsui, H., M. Kasao, and S. Imamura. High Performance Liquid Chromatographic Determination of Hippuric Acid in Human Urine, J. Chromatog., 45, 231 (1978).
[2] Sollenberg, J. and A. Baldesten. Isotachophoretic Analysis of Mandelic Acid, Phenylglyoxylic Acid, Hippuric Acid and Methyl Hippuric Acid in Urine After Occupational Exposure to Styrene, Toluene and/or Xylene, J. Chromatog., 132, 469 (1977).
[3] Tardif and Brodeur, J. Anal. Tox., 13, 313 (1985).
[4] Tietz, N.W. Fundamentals of Clinical Chemistry, 2nd ed., pp. 994-999, W.B. Saunders Co., Philadelphia, PA (1976).
[5] Lauwerys, R.R. Industrial Chemical Exposure: Guidelines for Biological Monitoring, Biomedical Publications, Davis, CA, pp. 57-69 (1983).

METHOD WRITTEN BY:

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